



MELAMINE IN MILK

PROFICIENCY TESTING

PROGRAM

APLAC T071

FINAL REPORT

26 November 2009



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Summary of Results

1. The objective of this proficiency test program (APLAC T071) was to evaluate the capability of participants on the quantitative analysis of melamine in milk. Two lyophilized test materials (Sample I and II) containing melamine at different concentrations were given to participants and they were instructed to reconstitute the dried samples to liquid milk with distilled water prior to analysis.
2. A total of 76 laboratories from 28 economies registered the program and 68 laboratories returned the results to the organizers within the scheduled timeline. An interim report containing participants' data and the respective z-score results was issued to participants and their ABs on 30 September 2009. Participants were requested to confirm the correctness of their submitted analytical data (before 23 October 2009) prior to the issuance of the Final Report by the organizers.
3. The exact quantities of melamine that gravimetrically spiked into material were used as the assigned reference values. The performance standard deviation was determined by Horwitz equation, and z-score was used to assess the performance of participants in this program.
4. Summary of participants' results:

	Sample I	Sample II
Data submitted	68	68
Valid numeric data	67	67
Robust mean (mg kg ⁻¹)	1.120	2.286
Median (mg kg ⁻¹)	1.090	2.260
Range (mg kg ⁻¹)	0.286 – 2.02	0.146 – 4.95
Between-laboratory variation	18.1%	13.9%
Horwitz SD (%)	15.5%	13.8%

5. Summary of participants' z-scores:

Performance	Number of participants (Percent)	
	Sample I	Sample II
$ z \leq 2$	58 (86.6%)	57 (85.1%)
$2 < z < 3$	5 (7.4%)	3 (4.5%)
$ z \geq 3$	4 (6.0%)	7 (10.4%)
Total:	67 (100%)	67 (100%)



1. Introduction

- 1.1. Melamine (1,3,5-triazine-2,4,6-triamine, or $C_3H_6N_6$) was first synthesized in 1834 and had later been found to have extensive uses in the industry. With high nitrogen content in the melamine molecule, the chemical could be unethically added to food products in order to increase the apparent protein content. Conventional analytical methods such as the Kjeldahl test which measures only the amount of nitrogen in samples and correlates the values as the protein content. As such, the nitrogen measurements can be misled by spiking samples with nitrogen-rich compounds like melamine. Specific analytical methods such as GC-MS, LC-MS/MS, CE and ELISA have recently been used in the determination of melamine in food matrices in most of the food testing laboratories.
- 1.2. With limited external quality assessments available to verify the measurement capability of those analytical methods for melamine, a proficiency testing program (APLAC T071) on the quantitative analysis of melamine in milk was proposed to the Asia-Pacific Laboratory Accreditation Co-operation (APLAC) PT Committee in May 2009. The program was organised by Hong Kong Accreditation Service (HKAS) in collaboration with the Hong Kong Government Laboratory (HKGL), under the auspices of APLAC. It is the second APLAC PT programme for melamine analysis this year. The first one “Melamine in fish feed (APLAC T069)” was completed in August 2009.
- 1.3. APLAC T071 commenced in February 2009 and anticipated to be completed in November to December 2009 (TABLE I). A total of 76 laboratories from 28 economies registered to participate in the program and 68 of them returned their results on or before the deadline (TABLE II). Each participant was confidentially assigned with a unique laboratory code (from 1 to 68).

2. Objectives

- 2.1. The main objective of this program was to evaluate the capability of participants on the quantitative analysis of melamine in milk with respect to the reference values.
- 2.2. Test results from participants were evaluated according to the statistical techniques outlined in ISO13528:2005^{9.1} “Statistical methods for use in proficiency testing by inter-laboratory comparisons” and in the international harmonized protocol for the proficiency testing



of analytical chemistry laboratories^{9,2}. z-Score was used as a numerical indicator to assess individual participant's proficiency relative to the reference values in the program.

3. Test Materials

- 3.1. About 20 litres of skimmed milk were purchased from a local supermarket and the samples were screened to be free of melamine. The samples were divided into two portions and labelled as Sample I and Sample II. Fats in the milk samples were removed using high speed centrifugation. The liquid phase was cautiously decanted into beakers and the total weight of the skimmed milk in both Sample I and II were weighed and recorded.
- 3.2. Two standard solutions of melamine (A and B) were gravimetrically prepared by dissolving appropriate quantities of neat melamine (purity = 99% which was validated against an independent source) in 10% v/v methanol as follows:

Standard Solution	Neat Melamine (g)	10% Methanol (g)	Total Weight (g)	Conc. of Melamine (mg kg ⁻¹)
A	0.0250	100.0091	100.0341	247.416
B	0.0402	100.0073	100.0475	397.791

- 3.3. The two milk samples as described in clause 3.1. were gravimetrically spiked with the melamine standard solutions in order to prepare nominal concentrations of melamine at approximately 1 and 2.5 mg kg⁻¹ as follow:

Sample	Wt. of milk (g)	Wt. of melamine spiked (g)	Total wt. (g)	Nominal conc. of melamine (mg kg ⁻¹)
I	8965.367	43.0190 of std. soln. A	9008.4	1.182
II	10043.326	64.472 of std. soln. B	10107.8	2.537

Sample mixtures were thoroughly stirred in low speed mixer for 30 min at 4 °C.

- 3.4. Both Sample I and II were kept in a freezer overnight before lyophilized at about -50°C and <100mT.



- 3.5. Conversion factor (CF) of the liquid milk to dried powder during lyophilisation was preliminarily determined using 10 g of samples ($n = 5$) and was found to be $7.91 \pm 0.075\%$. It means that 10 g of liquid milk will give 0.791 g of lyophilised milk during the process.

No.	Wt. of liq. milk (g)	Wt. of dish (g)	Total wt. after drying (g)	Wt. of dried powder (g)	CF (%)
1	10.081	116.142	116.934	0.792	7.86
2	10.029	120.483	121.281	0.798	7.96
3	10.029	116.44	117.223	0.783	7.81
4	10.026	119.638	120.437	0.799	7.97
5	10.037	117.031	117.831	0.800	7.97
				Mean:	7.91
				SD:	0.075
				RSD (%):	0.9535

- 3.6. Lyophilized samples were ground to fine powder by a high speed blender and sieved through 200 μm sieves. All batches of fine powder ($< 200 \mu\text{m}$) collected were mixed in a 3-dimensional mixer and the homogeneity status of mixing was monitored by taking random portions from the samples at regular intervals. Finally, the homogenized fine powder was packed into cleaned and nitrogen-flush amber glass bottles, each in 5 g portion and more than 100 bottles for each sample were prepared. All samples were disinfected by γ -irradiation at a dose of about 10 kGy dose and stored at room temperature before shipment.
- 3.7. Homogeneity (performed in April 2009) and stability (in July and October 2009) of the samples were determined in accordance with the procedures stipulated in APPENDICES I and II. Results indicated the samples were suitable to be used as the test materials for the program.
- 3.8. Test samples together with “Instructions for Accreditation Bodies”, “Instructions for Participating Laboratories”, “Receipt Form”, “Reconstitution Procedure” and “Result Proforma” were sent to participating laboratories or via their accreditation bodies (AB) in July 2009. A specimen copy of these instructions is given in APPENDICES III to VIII. All AB were requested to follow the procedures stipulated in “Instructions to Accreditation Bodies” and to distribute the sample(s) and the necessary documents to their nominated laboratories as soon as possible. As requested by some participating AB, their nominated



laboratories received the test samples and the documents directly from the organizers.

- 3.9. Participating laboratories were instructed to reconstitute the lyophilized test samples to liquid milk with distilled water in accordance with the procedure stipulated in the given “Reconstituted Procedure”.

4. Test Required

- 4.1. Participants were provided with two bottles of 5 g of lyophilized milk powder (labelled as Sample I and Sample II) and were required to determine the concentration of melamine (mg kg^{-1}) in the test samples by their appropriate methods. Participants were instructed to reconstitute the powder to liquid milk by adding 9.20 g of distilled water into 0.80 g powder. The reconstitution weighing process must be accurate.
- 4.2. Participants were asked to report the results in mg kg^{-1} to three significant figures or three decimal places, the expanded measurement uncertainty and technical information of the method used.
- 4.3. All analytical results and information should be reported in the Result Proforma provided.

5. Estimation of Reference Values and their Uncertainty

- 5.1. Reference values (x_{ref}) were calculated from the nominated concentrations in Cl. 3.3. and the CF in Cl. 3.5.

$$\begin{aligned}x_{\text{ref}} \text{ for Sample I} &= 1.182 \times (8.0/7.91) \\&= 1.182 \times 1.011 \\&= \underline{1.195 \text{ mg kg}^{-1}}\end{aligned}$$

$$\begin{aligned}x_{\text{ref}} \text{ for Sample II} &= 2.537 \times (8.0/7.91) \\&= 2.537 \times 1.011 \\&= \underline{2.565 \text{ mg kg}^{-1}}\end{aligned}$$

- 5.2. The uncertainty of the reference values included the effect of purity of melamine standard, variability of between-bottle in the homogeneity test, variability of lyophilisation and weighing errors. Expanded uncertainty of the reference values at $k = 2$ & confidence interval of 95% was estimated to be 0.045 mg kg^{-1} (3.8%) for Sample I and 0.113 mg kg^{-1} (4.4%) for Sample II respectively.



6. Statistical Evaluation

6.1. Participants' test results

6.1.1. Test results received from participants are presented in TABLE III and IV. Their methodologies and techniques employed as well as their accreditation status are tabulated in TABLES V and VI.

6.2. Performance evaluation

6.2.1. Performance of each participant was evaluated by means of a performance index, z-score, which was calculated as:

$$Z = \frac{X_i - X_{\text{ref}}}{\sigma}$$

where x_i = reported mean of individual participant
 x_{ref} = reference value
 σ = standard deviation (in mg kg⁻¹) estimated from Horwitz equation

6.2.2. z-Score is commonly interpreted as follows:

- (i) $|z| \leq 2$: Satisfactory
- (ii) $2 < |z| < 3$: Questionable
- (iii) $|z| \geq 3$: Unsatisfactory

6.2.3. Participants having $|z| \geq 3$ are regarded as giving unsatisfactory results and they should investigate the cause and take necessary follow up actions. Participants having z-score(s) in the range $2 < |z| < 3$ are encouraged to review their results.

7. Results and Discussions

7.1. All of the 68 participants submitted results for both samples. Only one participant (Lab. #17) reported melamine concentration was below the detection limit at 2.0 mg kg⁻¹. Distribution of participants' data was displayed graphically in GRAPHS I and II. The concentration was ranging from 0.286 – 2.02 mg kg⁻¹ (Sample I) and 0.146 – 4.95 mg kg⁻¹ (Sample II). The robust mean values were 1.120 and 2.285 mg kg⁻¹ respectively for Sample I and II. Between-laboratory variations were in good agreement with the target performance deviation estimated using Horwitz equation.

Summary of participants' results is as follows:

	Sample I	Sample II
Data submitted	68	68
Valid numeric data	67	67
Robust mean (mg kg ⁻¹)	1.120	2.285
Median (mg kg ⁻¹)	1.090	2.260
Range (mg kg ⁻¹)	0.286 – 2.02	0.146 – 4.95
Between-laboratory variation	18.1%	13.9%
Horwitz SD (%)	15.5%	13.8%

- 7.2. z-Scores of all participants are tabulated in TABLE VII and illustrated in GRAPHS III and IV. The overall performance, in terms of z-score achievement, of participants is summarized as follow:

Performance	Number of participants (Percent)	
	Sample I	Sample II
$ z \leq 2$	58 (86.6%)	57 (85.1%)
$2 < z < 3$	5 (7.4%)	3 (4.5%)
$ z \geq 3$	4 (6.0%)	7 (10.4%)
Total:	67 (100%)	67 (100%)

In brief, 7 participants (Lab. #15, 31, 41, 49, 51, 54 and 61) or 10.4% of the population were identified to report one or more unsatisfactory results ($|z| \geq 3$) and 11 out of the 134 results (8.2 %) were identified as unsatisfactory results.

- 7.3. Overview of participants' methodologies and performance (TABLES V and VI):

7.3.1. LC-MS/MS technique (75%) was commonly used by participants, followed by GC-MS (13.2%), LC-UV (7.4%) and ELISA (4.4%).

7.3.2. Majority of the LC-MS/MS users (33 out of 51) employed hydrophilic interaction liquid chromatography (HILIC) columns and the remaining used either C₈, C₁₈ or amino columns for their chromatographic separation. Retention of polar molecules such as melamine in HILIC columns improves with the increased polarity of organic solvent in the mobile phase. Such

chromatographic separation principle was exercised well as all LC users in this program used the mobile phase containing high proportion of acetonitrile in acidic medium. LC-UV users employed C₈ or C₁₈ as their analytical columns, whereas GC-MS users employed relatively polar GC columns such as DB-5 for chromatographic separation of the derivatized melamine.

- 7.3.3. With the high polarity of melamine, participants preferred to use polar organic solvents (acetonitrile/methanol; or acidified acetonitrile/methanol or acid such as diluted hydrochloric acid, formic acid, acetic acid, trifluoroacetic acid, trichloroacetic acid) in their extraction with shaking, sonication and agitation. Six GC-MS participants employed USFDA method and used alkaline medium, diethylamine and aqueous acetonitrile for extracting melamine. Duration of extraction varied from 1 min to 240 min. The recovery of melamine was generally good. Forty two participants claimed it was greater than 80%. However, Lab.#28 gave an extremely low recovery results probably due to short extraction time (1 min.). Thirty participants used solid phase extraction (eg. cationic exchange columns) as the sample clean up procedure.
- 7.3.4. Unsatisfactory performance was found in LC-MS/MS (3 out of 51); GC-MS (3 out of 9) and in LC-UV (1 out of 5) methods. Apart from one questionable result in Sample II, the three ELISA participants performed satisfactorily. Furthermore, all the twenty nine LC-MS/MS users (Lab.#3, 6-14, 18-21, 23-25, 28-29, 33-35, 37, 43, 46, 52, 55, 60, 63 and 66) and four GC-MS users (Lab.#1, 47, 53 and 65) who employed isotope internal standard (¹³C₃¹⁵N₃ melamine, ¹³C₃ melamine or ¹⁵N₃ melamine) in the analysis obtained |z|-score ≤ 2. (Note: Lab.#15, 22 and 48 claimed to use internal standard but they did not specify the nature)
- 7.3.5. A comparison of the four major techniques indicated that results from LC-MS/MS were close to the reference values. Precision, in terms of RSD, of ELISA and LC-MS/MS were found to be better in this program.

Technique	Sample I		Sample II	
	Mean (mg kg ⁻¹)	RSD	Mean (mg kg ⁻¹)	RSD
ELISA (n = 3)	1.014	18.0%	2.145	19.4%
GC-MS (n = 9)	1.019	50.2%	2.271	59.4%
LC-MS/MS (n = 51)	1.165	16.6%	2.278	22.6%
LC-UV (n = 5)	0.935	33.3%	1.965	26.7%



- 7.3.6. Amongst the twenty six participants who claimed their methods were accredited, only one participant (Lab.#15) had unsatisfactory results. For the thirty eight non-accredited participants, six (Lab.#31, 41, 49, 51, 54 and 61) had one or more than one unsatisfactory results. In view of the above results, the performance of accredited laboratories for analysing melamine in milk at two concentrations was better in this program.
- 7.3.7. Large variation of reported MU was found amongst participants (TABLE III and IV) and the coverage factor (k) was mostly at 2. For example, the relative MU of Sample II from Lab.#15 was 0.08% but from Lab.#61 was 253%. The median relative MU was 15% and 10% respectively for Sample I and II. Nine participants did not report MU. This indicated that some participants did not fully comprehend with the uncertainties associated with their testing and therefore possessed difficulties in giving a correct MU estimate. Although MU data were not used in the performance assessment in this program, participants should be capable to present correct and sensible MU estimation as it is one of the requirements in the ISO/IEC 17025. Distribution of participants' MU is summarized below.

Report MU Range	Sample I	Sample II
	No. of Participants	No. of Participants
< 5%	9	14
5% - 10%	8	12
10% - 20%	23	20
20% - 30%	15	6
> 30%	4	7
Median MU	15%	10%

For those using accredited methods ($n = 19$), the reported MU were also found to be varied (GRAPH V). Three of those accredited laboratories (Lab.#5, 26 & 58) did not report the MU. Further training in this area is recommended and ABs should also be responsible to ensure that MU estimation from their laboratories is properly performed during the assessments.



8. Remarks

- 8.1. If participants have further comments or disagree with the assessment, please contact the organizers in the following addresses:

Yiu-chung WONG
Government Laboratory,
7/F. Homantin Government Offices,
88 Chung Hau Street, Kowloon, Hong Kong.
email: ycwong@govtlab.gov.hk

or

W. W. WONG
Hong Kong Accreditation Service,
36/F, Immigration Tower,
7 Gloucester Road, Wanchai, Hong Kong
email: wwwong@itc.gov.hk

- 8.2. Contributions and efforts from all participating ABs and laboratories to this program are gratefully noted. Special thanks are also extended to the APLAC PT Committee, the Government Chemist of Government Laboratory and HKAS Executive for their support to the program.

9. References

- 9.1. ISO 13528:2005. Statistical methods for use in proficiency testing by interlaboratory comparisons, **2005**, Geneva, Switzerland.
- 9.2. M. Thompson, S.L. Ellison, R. Wood. The International harmonized protocol for the proficiency testing of analytical chemistry laboratories (IUPAC technical report), *Pure Appl. Chem.*, **2006**, 78: 146-196.
- 9.3. M. Chan, C.K. Lo, L.S. Cheng, T.C. Cheung and Y.C. Wong. "Evaluation of testing capabilities for the determination of melamine in milk through an interlaboratory comparison program during the melamine crisis", *Food Addit. Contam.*, **2009**, 26:1450-1458.

**TABLE I: Timeline of the Program**

EVENT	SCHEDULE
Preparation of sample	Feb – Apr 2009
Homogeneity testing	Apr 2009
Submission of proposal to APLAC PT Committee for approval	May 2009
Stability testing	Jun – Oct 2009
Invitation of participants	June – Jul 2009
Dispatch of samples	Jul 2009
Submission of results	Sept 2009
Statistical analysis of results	Oct 2009
Interim report	Oct 2009
Submission of draft report to PTAB / APLAC PT Committee	Nov 2009
Approval of draft report by PTAB / APLAC Proficiency Committee	Nov – Dec 2009
Distribution of final report	Nov – Dec 2009

**TABLE II: Geographic Distribution of Participating Laboratories**

No.	ECONOMIES	PARTICIPANTS ENROLLED	RETURNED RESULTS
1	Australia	4	3
2	Brazil	2	2
3	Brunei Darussalam	1	1
4	Canada	4	4
5	Chile	1	0
6	China	5	5
7	Costa Rica	1	0
8	Croatia	1	0
9	Czech Republic	2	2
10	Denmark	1	1
11	Estonia	1	1
12	France	1	1
13	Germany	7	7
14	Hong Kong	8	7
15	Indonesia	4	4
16	Jamaica	1	1
17	Japan	5	5
18	Korea	1	1
19	Malaysia	1	1
20	Netherland	1	1
21	New Zealand	4	4
22	Philippines	2	1
23	Poland	1	1
24	Sri Lanka	1	0
25	Switzerland	4	3
26	Thailand	5	5
27	USA	3	3
28	Vietnams	4	4
	Total:	76	68

**TABLE III: Participants' Results for Melamine in Sample I**

Lab. Code	Concentration (mg kg ⁻¹)				Expanded Measurement Uncertainty	
	Data 1	Data 2	Data 3	Mean	± mg kg ⁻¹	Coverage factor
1	1.02	1.07	1.04	1.04	0.154	2
2	1.068	1.061	1.099	1.076	0.154	2
3	1.20	1.07	1.06	1.11	0.22	2
4	1.438	1.574	1.180	1.397	0.399	2
5	1.08	---	---	1.08	---	---
6	1.15	0.96	1.02	1.04	0.312	2
7	1.056	1.095	---	1.08	0.54	2.179
8	1.24	1.21	1.11	1.19	0.12	---
9	0.905	---	---	0.905	0.45	2
10	1.21	1.26	1.24	1.24	0.248	2
11	0.589	0.842	---	0.716	---	---
12	1.072	1.146	1.102	1.107	0.135	2
13	1.301	1.308	1.337	1.316	0.038	2
14	1.051	1.053	1.112	1.072	0.297	2
15	0.554	0.546	0.568	0.556	0.001	0.001
16	1.087	1.034	1.058	1.051	0.12	2
17	≤2.0	≤2.0	≤2.0	≤2.0	0.06	2
18	1.067	1.157	0.967	1.06	0.16	2
19	1.134	1.131	1.144	1.136	0.238	2
20	1.03	1.04	1.06	1.04	0.08	---
21	1.08	1.06	---	1.07	0.12	2
22	1.185	1.200	1.114	1.166	0.310	---
23	1.09	1.11	---	1.10	0.1	2
24	1.12	1.16	1.14	1.14	0.14	2
25	1.17	1.16	1.20	1.18	0.22	2.064
26	1.5	---	---	1.5	---	---
27	1.03	1.04	1.04	1.03	0.19	2
28	1.0	1.0	1.0	1.0	0.0	2
29	1.10	1.04	1.10	1.08	0.08	2
30	1.2	1.2	1.3	1.2	0.09	2
31	0.310	0.280	0.275	0.288	0.042	2
32	1.33	1.37	1.19	1.30	0.189	2
33	1.07	0.92	1.01	1.01	0.12	2
34	1.28	1.34	1.45	1.36	0.24	2

Notes: "—" denotes data or information not provided. Expanded measurement uncertainty was not used for performance assessment.

**TABLE III (Cont'd): Participants' Results for Melamine in Sample I**

Lab. Code	Concentration (mg kg ⁻¹)				Expanded Measurement Uncertainty	
	Data 1	Data 2	Data 3	Mean	± mg kg ⁻¹	Coverage factor
35	1.26	1.13	---	1.20	0.3	2.83
36	1.092	1.093	1.096	1.082	0.211	-
37	1.14	1.05	1.10	1.10	0.24	2
38	1.20	1.17	1.22	1.20	0.45	2
39	1.08	1.11	1.12	1.10	0.1	2
40	1.04	1.07	1.00	1.04	---	---
41	2.02	---	---	2.02	---	---
42	1.28	1.30	1.05	1.21	0.28	2
43	1.07	1.16	1.13	1.12	0.34	2
44	0.88	0.87	0.89	0.88	0.1	---
45	0.988	0.651	---	0.819	0.058	2
46	1.01	1.06	---	1.04	0.2656	2
47	0.990	1.000	0.992	0.994	0.03	2
48	0.902	0.909	---	0.906	0.05	-
49	1.60	1.50	---	1.55	0.23	2
50	0.874, 0.876	0.919, 0.913	---	0.896	0.024	2
51	0.586	0.614	---	0.600	---	---
52	0.935	0.905	0.927	0.922	0.01	---
53	1.127	1.012	1.130	1.090	0.120	2
54	0.789	0.755	---	0.772	0.227	2
55	1.21	1.39	1.36	1.32	0.289	3
56	1.124	0.987	0.973	1.028	0.146	2
57	1.68	1.64	1.44	1.58	0.40	3.182
58	1.124	1.239	---	1.182	---	---
59	1.678	1.761	1.495	1.643	0.272	2
60	1.06	1.07	1.06	1.06	0.05	2
61	1.61	1.63	1.61	1.62	0.37	2
62	0.93	1.70	1.51	1.38	---	---
63	1.29	1.32	1.39	1.3	0.3	2
64	1.402	1.402	1.404	1.403	0.0674	2
65	1.27	1.28	1.27	1.27	0.18	2
66	0.999	0.995	1.071	1.022	0.090	2
67	1.39	1.44	1.42	1.42	0.05	2
68	0.880	---	---	0.880	---	---

Notes: "—" denotes data or information not provided. Expanded measurement uncertainty was not used for performance assessment.

**TABLE IV: Participants' Results for Melamine in Sample II**

Lab. Code	Concentration (mg kg ⁻¹)				Expanded Measurement Uncertainty	
	Data 1	Data 2	Data 3	Mean	± mg kg ⁻¹	Coverage factor
1	2.15	2.23	2.18	2.19	0.154	2
2	2.510	2.503	2.507	2.507	0.352	2
3	2.30	2.28	2.21	2.26	0.45	2
4	1.896	2.202	1.853	1.984	0.381	2
5	1.98	---	---	1.98	---	---
6	2.37	2.04	2.28	2.23	0.669	2
7	2.167	2.335	---	2.25	1.13	2.179
8	2.22	2.50	2.38	2.37	0.24	-
9	1.96	---	---	1.96	0.98	2
10	2.74	2.70	2.71	2.71	0.542	2
11	1.853	1.475	---	1.664	---	---
12	2.377	2.451	2.376	2.401	0.263	2
13	2.713	2.650	2.688	2.684	0.064	2
14	2.108	2.202	2.228	2.179	0.604	2
15	1.305	1.153	1.123	1.194	0.001	0.001
16	2.384	2.110	2.229	2.241	0.276	2
17	≤2.0	≤2.0	≤2.0	≤2.0	0.06	2
18	2.418	2.083	2.164	2.22	0.33	2
19	2.410	2.420	2.448	2.426	0.508	2
20	2.29	2.27	2.27	2.27	0.18	---
21	2.18	2.28	---	2.23	0.23	2
22	2.573	2.647	2.436	2.552	0.679	---
23	2.28	2.13	---	2.23	0.1	2
24	2.35	2.43	2.36	2.38	0.23	2
25	2.30	2.46	2.45	2.40	0.46	2.064
26	2.2	---	---	2.2	---	---
27	2.12	2.27	2.16	2.18	0.39	2
28	2.2	2.2	2.3	2.2	0.1	2
29	2.30	2.32	2.28	2.30	0.08	2
30	2.5	2.6	2.5	2.5	0.09	2
31	0.329	0.256	0.256	0.280	0.042	2
32	2.54	2.47	2.44	2.48	0.103	2
33	2.28	1.99	2.07	2.11	0.22	2
34	2.63	2.67	2.88	2.73	0.49	2

Notes: "—" denotes data or information not provided. Expanded measurement uncertainty was not used for performance assessment.

**TABLE IV (Cont'd): Participants' Results for Melamine in Sample II**

Lab. Code	Concentration (mg kg ⁻¹)				Expanded Measurement Uncertainty	
	Data 1	Data 2	Data 3	Mean	± mg kg ⁻¹	Coverage factor
35	2.48	2.37	---	2.43	0.2	2.83
36	2.146	2.289	2.162	2.199	0.429	---
37	2.38	2.39	2.21	2.33	0.50	2
38	2.46	2.45	2.47	2.46	0.93	2
39	2.32	2.33	2.38	2.34	0.1	2
40	2.03	1.92	1.77	1.91	---	---
41	4.95	---	---	4.95	---	---
42	2.26	2.17	1.92	2.12	0.36	2
43	2.25	2.55	2.57	2.46	0.74	2
44	2.04	2.05	2.04	2.05	0.1	---
45	1.834	1.963	---	1.898	0.135	2
46	2.42	2.67	---	2.54	0.2656	2
47	2.551	2.609	2.603	2.580	0.25	2
48	2.140	2.150	---	2.15	0.13	-
49	1.20	1.06	---	1.13	0.17	2
50	1.89, 1.93	1.98, 1.97	---	1.94	0.04	2
51	1.18	1.30	---	1.24	---	---
52	2.017	2.047	1.996	2.018	0.02	---
53	2.293	2.197	2.370	2.287	0.115	2
54	0.733	0.812	0.682	0.742	0.227	2
55	2.05	2.27	2.45	2.26	0.601	3
56	2.678	2.669	2.620	2.656	0.146	2
57	3.49	3.39	3.70	3.53	0.50	3.182
58	2.653	2.599	---	2.626	---	---
59	3.032	2.956	2.904	2.964	0.128	2
60	2.27	2.30	2.26	2.28	0.11	2
61	0.122	0.139	0.176	0.146	0.37	2
62	2.22	2.70	2.72	2.55	---	---
63	2.47	2.58	2.58	2.5	+/- 0.2	2
64	2.483	2.521	2.508	2.504	0.1202	2
65	2.86	2.66	2.74	2.75	0.39	2
66	2.189	2.200	2.145	2.178	0.112	2
67	3.35	3.39	3.35	3.36	0.24	2
68	2.03	---	---	2.03	---	---

Notes: "—" denotes data or information not provided. Expanded measurement uncertainty was not used for performance assessment.

**TABLE V: Instruments and Conditions Used by Participants**

Lab Code	Analytical Instrument	Analytical Column	Mobile phase / Carrier gas	Calibration Method	Internal standard
1	GC-MS	HP-5MS	He	Matrix Cal.	¹³ C ₃ ¹⁵ N ₃ melamine
2	LC-MS/MS	ZIC HILIC	NH ₄ Ac, ACN	---	No
3	LC-MS/MS	C8	NH ₄ Ac, ACN	---	¹³ C ₃ ¹⁵ N ₃ melamine
4	LC-MS/MS	ZIC HILIC	NH ₄ Ac, ACN	Ext. Cal.	No
5	LC-MS/MS	HILIC	NH ₄ Ac, ACN	---	No
6	LC-MS/MS	Hypercarb	NH ₄ Fo, ACN	---	¹⁵ N ₃ melamine
7	LC-MS/MS	HILIC	NH ₄ Ac, ACN	Ext. Cal.	labelled melamine
8	LC-MS/MS	Hypersil APS-2	NH ₄ Ac, ACN	Std. Add.	¹³ C ₃ ¹⁵ N ₃ melamine
9	LC-MS/MS	HILIC	NH ₄ Ac, ACN	Std. Add.	¹³ C ₃ ¹⁵ N ₃ melamine
10	LC-MS/MS	HILIC	-	Matrix Cal.	¹³ C ₃ ¹⁵ N ₃ melamine
11	LC-UV	C18	NH ₄ Ac, THF	1p Cal.	No
12	LC-MS/MS	ZIC HILIC	NH ₄ SO ₄ , ACN	Ext. Cal.	¹³ C ₃ ¹⁵ N ₃ melamine
13	LC-MS/MS	Amide-80	NH ₄ Ac, ACN	---	¹³ C ₃ ¹⁵ N ₃ melamine
14	LC-MS/MS	C18	NH ₄ Ac, ACN	---	¹³ C ₃ ¹⁵ N ₃ melamine
15	GC-MS	DB5	He	1p Cal.	Yes
16	LC-MS/MS	Silica	NH ₄ Fo, ACN	---	No
17	GC-MS	DB-5MS		---	No
18	LC-MS/MS	HILIC	NH ₄ Ac, ACN	Ext. Cal.	¹³ C ₃ ¹⁵ N ₃ melamine
19	LC-MS/MS	HILIC	NH ₄ Fo, ACN	Ext. Cal.	¹³ C ₃ ¹⁵ N ₃ melamine
20	LC-MS/MS	ZB5MS	He	Matrix Cal.	¹³ C ₃ ¹⁵ N ₃ melamine
21	LC-MS/MS	HILIC	---	---	labelled melamine
22	LC-MS/MS	HILIC	NH ₄ Fo, ACN	Matrix Cal.	Yes
23	UPLC-MS/MS	HILIC	NH ₄ Ac, ACN	---	¹³ C ₃ ¹⁵ N ₃ melamine
24	LC-MS/MS	ZIC-HILIC	NH ₄ Fo, ACN	Std. Add.	¹⁵ N ₃ melamine
25	LC-MS/MS	HILIC	NH ₄ Fo, ACN	---	¹³ C-melamine
26	LC-MS/MS	C18	NH ₄ Ac, ACN	Std. Add.	No
27	LC-MS/MS	HILIC	NH ₄ Ac, ACN	---	No
28	LC-MS/MS	HILIC	NH ₄ Ac, ACN	Ext. Cal.	¹³ C ₃ ¹⁵ N ₃ melamine
29	LC-MS/MS	C18	NH ₄ Ac, ACN	Ext. Cal.	¹⁵ N ₃ melamine
30	LC-MS/MS	HILIC	NH ₄ Fo, ACN	1p Cal.	No
31	GC-MS	DB 5	He	---	2,6-diamino-4-chloropyrimidine
32	LC-MS/MS	HILIC	NH ₄ Ac, ACN	---	No
33	LC-MS/MS	HILIC	NH ₄ Fo, ACN	Ext. Cal.	¹³ C ₃ ¹⁵ N ₃ melamine
34	LC-MS/MS	HILIC	NH ₄ Fo, NH ₄ Ac, ACN	---	¹³ C ₃ ¹⁵ N ₃ melamine

Notes: "—" denotes data or information not provided; ACN: Acetonitrile; NH₄Ac: Ammonium acetate; NH₄Fo: Ammonium formate; NH₄SO₄: Ammonium sulphate; THF: Tetrahydrofuran; 1p Cal.: One-point calibration; Ext. Cal. External calibration; Std. Add.: Standard addition

**TABLE V (Cont'd): Instruments and Conditions Used by Participants**

Lab Code	Analytical Instrument	Analytical Column	Mobile phase / Carrier gas	Calibration Method	Internal standard
35	LC-MS/MS	HILIC	NH ₄ Fo, ACN	---	¹³ C ₃ melamine
36	LC-MS/MS	HILIC	H ₂ O, ACN	1p Cal.	No
37	LC-MS/MS	Reprosil polyamine	AcOH, ACN	---	¹³ C ₃ ¹⁵ N ₃ melamine
38	LC-MS/MS	Amide-80	NH ₄ Fo, ACN	Ext. Cal.	No
39	LC-UV	Capcell CR	NH ₄ Ac, ACN	Ext. Cal.	No
40	ELISA	NA	NA	---	NA
41	GC-MS	DB-XLB	He	1p Cal.	No
42	LC-MS/MS	HILIC	NH ₄ Ac, ACN	---	BZN
43	LC-MS/MS	C18	NH ₄ Ac, ACN	---	labelled melamine
44	LC-MS/MS	C18	H ₂ O, ACN	---	---
45	ELISA	NA	NA	---	NA
46	LC-MS/MS	Polar RP	NH ₄ Ac, ACN	No	¹³ C ₃ ¹⁵ N ₃ melamine
47	GC-MS	DB-5	He	No	¹⁵ N ₃ melamine
48	LC-MS/MS	C18	NH ₄ Ac, MeOH	Std. Add.	Yes
49	LC-MS/MS	ZIC-HILIC	NH ₄ Fo, ACN	---	No
50	GC-MS	DB35 and C18	He	Ext. Cal.	No
51	LC-UV	Zorbax SB C18	PO ₄ buffer, ACN	No	No
52	LC-MS/MS	HILIC	NH ₄ Ac, ACN	No	¹³ C ₃ ¹⁵ N ₃ melamine
53	GC-MS	DB 5	He	No	¹⁵ N ₃ melamine
54	LC-MS/MS	ZIC-HILIC	NH ₄ Ac, ACN	Ext. Cal.	No
55	LC-MS/MS	ZIC-HILIC	NH ₄ Ac, ACN	Ext. Cal.	¹³ C ₃ melamine
56	LC-MS/MS	HILIC	NH ₄ Ac, ACN	No	d ₅ Chloramphenicol
57	LC-MS/MS	ZIC-HILIC	NH ₄ Ac, ACN	Matrix Cal.	No
58	ELISA	NA	NA	Ext. Cal.	NA
59	LC-MS/MS	HILIC	NH ₄ Fo, ACN	---	No
60	LC-MS/MS	HILIC	NH ₄ Ac, ACN	---	¹³ C ₃ ¹⁵ N ₃ melamine
61	LC-MS/MS	HILIC	NH ₄ Ac, ACN	Ext. Cal.	No
62	LC-UV	C8	SOS, CA, ACN	---	No
63	LC-MS/MS	ZIC-HILIC	NH ₄ Ac, ACN	Ext. Cal.	¹⁵ N ₃ melamine
64	LC-MS/MS	Zorbak RX-SIL	NH ₄ Ac, ACN	---	No
65	GC-MS	DB-5	He	---	¹³ C ₃ ¹⁵ N ₃ melamine
66	LC-MS/MS	Amino	H ₂ O, AcOH	---	¹⁵ N ₃ melamine
67	LC-MS/MS	---	H ₂ O, ACN	Std. Add.	No
68	LC-UV	---	PO ₄ buffer, ACN	Ext. Cal.	No

Notes: "—" denotes data or information not provided; "NA" denotes not applicable; ACN: Acetonitrile; AcOH: Acetic acid; CA: Citric acid; MeOH: Methanol; NH₄Ac: Ammonium acetate; NH₄Fo: Ammonium formate; NH₄SO₄: Ammonium sulphate; PO₄: Phosphate; SOS: sodium octane sulphonate; THF: Tetrahydrofuran; 1p Cal.: One-point calibration; Ext. Cal. External calibration; Std. Add.: Standard addition

**TABLE VI: Other Technical Information**

Lab Code	Extraction Method	Extraction Solvent	Clean-up Procedure	% Recovery	Method Accredited
1	Shaking, Ultrasonic, MCX	1%TCA, MeOH	MCX	99.29	No
2	Shaking, MCX	ACN, H ₂ O	MCX	98.26	No
3	Ultrasonic	ACN	MCX	---	No
4	Ultrasonic	ACN, H ₂ O	Dilution	60	No
5	Ultraturrax, Centrifugation	ACN, H ₂ O	SPE	80	Yes
6	Agitation, Shaking, Ultrasonic, SPE C18	H ₂ O	SPE	70	Yes
7	Shaking, ultrasonic, MCX	ACN, H ₂ O	MCX	100	Yes
8	Shaking, MCX	DCM	---	---	Yes
9	Shaking	ACN, H ₂ O	Liq-Liq partition	86	Yes
10	Shaking, MCX	ACN, HCl	---	111.5	No
11	Ultrasonic, SCX	5%TCA	SCX	---	No
12	Shaking, Ultrasonic	ACN, H ₂ O	Centrifuge	103.3	No
13	Shaking, centrifugation	ACN, H ₂ O	---	---	No
14	Shaking, MCX	ACN, H ₂ O, HCl	MCX	96	No
15	Ultrasonic, SPE C18	ACN, H ₂ O	SPE	91	Yes
16	SCX	2% TCA	SCX	100	No
17	Ultrasonic	DEA, ACN, H ₂ O	pyridine and Sylon BFT	---	---
18	Ultrasonic, MCX	1%TCA, ACN	MCX	96.5	No
19	Agitation, Shaking, Ultrasonic, MCX	ACN, H ₂ O	Membrane Filtration	87.7	Yes
20	Liq-liq. Extraction	ACN, H ₂ O	Filtration	100	Yes
21	---	---	---	---	---
22	Shaking	ACN, H ₂ O	MCX		No
23	Agitation, Shaking, Ultrasonic, MCX	ACN, H ₂ O	SPE	102	No
24	Shaking, Ultrasonic	FA, ACN, H ₂ O	Syringe filter	84.6-110	No
25	Shaking, Ultrasonic	ACN	No	84-86	Yes
26	Shaking	H ₂ O	Ultrafiltration	---	No
27	Liq-liq. Extraction	ACN, H ₂ O	No	90	No
28	Shaking, MCX	ACN, H ₂ O	MCX	23.4-24.6	Yes
29	Ultrasonic	1% TCA, ACN	---	99-103	No
30	Ultrasonic	ACN, H ₂ O	---	94-103	No
31	Ultrasonic	DEA, ACN, H ₂ O	---	---	No
32	MCX	H ₂ O	MCX	99.1	No
33	Liq-liq Extraction	ACN, H ₂ O	Centrifuge + filter	91	No
34	Shaking, Ultrasonic	TCA, ACN	Centrifuge + SPE	108	Yes

Notes: “---” denotes data or information not provided; “NA” denotes not applicable; “ACN” = acetonitrile; “BSTFA” = N,O-Bis(trimethylsilyl)trifluoroacetamide; “DEA” = diethylamine; “FA” = formic acid; “MeOH” = methanol; “TCA” = trichloroacetic acid; “TFA” = trifluoroacetic acid; “MCX, SCX” = cationic exchange columns; PSA: amines column

**TABLE VI (Cont'd): Other Technical Information**

Lab Code	Extraction Method	Extraction Solvent	Clean-up Procedure	% Recovery	Method Accredited
35	MCX	0.1M HCl	Centrifuge + SPE	100	Yes
36	Shaking	ACN, H ₂ O	Dilution	---	No
37	Ultrasonic	ACN, H ₂ O	MCX	106.1	No
38	Shaking	2.5% FA	Dilution	93-111	No
39	Ultrasonic	TCA, ACN	MCX	90	Yes
40	---	---	---	---	---
41	Agitation, Ultrasonic	DEA, ACN, H ₂ O	Centrifuge + filter	---	No
42	---	1% TFA	MCX	99-111	No
43	Agitation, Ultrasonic	ACN, H ₂ O, HCl	MCX	98	No
44	---	FA, H ₂ O	---	96-102	-
45	Suspension	---	---	101.4	No
46	Shaking	ACN, DCM, H ₂ O	SCX	75-102	Yes
47	Ultrasonic	1% TCA, MeOH	SCX	90-110	No
48	Shaking + heating	---	Dilution	89	Yes
49	Liq-Liq Extraction	FA, H ₂ O	Centrifuge + filter	---	No
50	Agitation, Shaking, Ultrasonic	DEA, ACN, H ₂ O	Centrifuge + filter	91.1	Yes
51	Liq-Liq. Extraction	NH ₄ OH, MeOH	SCX	90-95	No
52	Liq-Liq Extraction	TFA, MeOH	SCX	---	No
53	Ultrasonic	DEA, ACN, H ₂ O	Precipitation with ACN	73.2	No
54	Shaking	ACN:0.1N HCl	MCX	---	No
55	Agitation, centrifugation	ACN, H ₂ O	MCX	92.4-97.9	No
56	Agitation, Ultrasonic, Centrifugation	ACN	---	53.6	No
57	Shaking	FA, ACN, H ₂ O	Filter	30	No
58	---	MeOH, H ₂ O	---	---	Yes
59	Agitation	FA, ACN	Centrifuge + filter	94	Yes
60	Shaking, MCX	TCA, ACN	SPE	100.2	No
61	Ultrasonic + boil	ACN, H ₂ O	---	---	No
62	Liq-Liq. Extraction	ACN, H ₂ O	---	98-99	No
63	Shaking	ACN	MCX	98.8-106	Yes
64	MCX	FA, ACN	---	82	Yes
65	SPE	ACN	SCX	103.28	No
66	Ultrasonic	DEA, ACN, H ₂ O	Precipitation with ACN	73.2	No
67	---	TCA, H ₂ O	PSA	46-50	No
68	Liq-liq Extraction	TCA, ACN, H ₂ O	Centrifuge + filter	---	---

“---” denotes information was not provided by participants; “NA” denotes not applicable; “ACN” = acetonitrile; “BSTFA” = N,O-Bis(trimethylsilyl)trifluoro acetamide; “DEA” = diethylamine; “FA” = formic acid; “MeOH” = methanol; TCA” = trichloroacetic acid; “TFA” = trifluoroacetic acid; “MCX, SCX” = cationic exchange columns; PSA: amines column

**TABLE VII: z-Scores of Participants**

Lab. Code	z-Score	
	Sample I	Sample II
1	-0.84	-1.1
2	-0.64	-0.16
3	-0.46	-0.86
4	1.1	-1.6
5	-0.62	-1.7
6	-0.84	-0.95
7	-0.62	-0.89
8	-0.03	-0.55
9	-1.6	-1.7
10	0.24	0.41
11	-2.6	-2.6
12	-0.48	-0.46
13	0.66	0.34
14	-0.67	-1.1
15	-3.5	-3.9
16	-0.78	-0.95
17	NA	NA
18	-0.73	-1.0
19	-0.32	-0.39
20	-0.84	-0.84
21	-0.68	-0.95
22	-0.16	-0.04
23	-0.51	-0.95
24	-0.30	-0.52
25	-0.08	-0.47
26	1.7	-1.0
27	-0.89	-1.1
28	-1.1	-1.0
29	-0.62	-0.75
30	0.03	-0.18
31	-4.9	-6.5
32	0.57	-0.24
33	-1.0	-1.3
34	0.89	0.47

z-Scores ≥ 3 or ≤ -3 are bolded; NA: not applicable

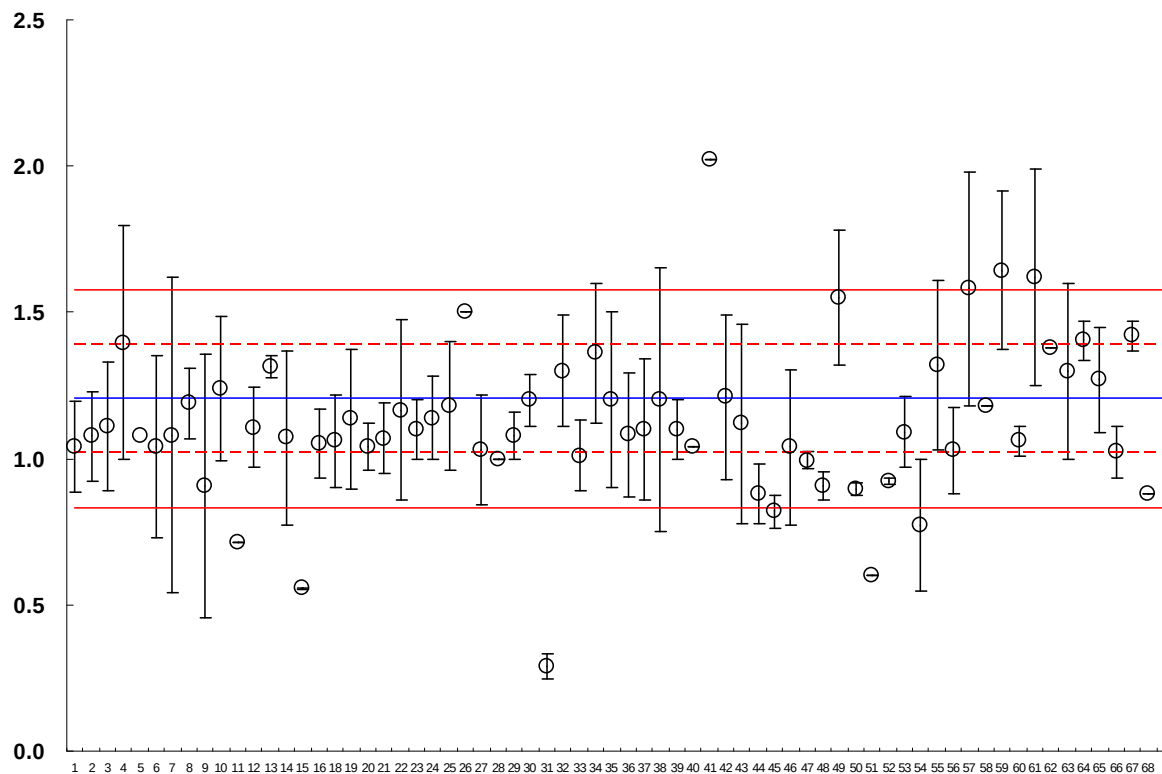
**TABLE VII (Cont'd): z-Scores of Participants**

Lab. Code	z-Score	
	Sample I	Sample II
35	0.03	-0.38
36	-0.61	-1.0
37	-0.51	-0.67
38	0.03	-0.30
39	-0.51	-0.64
40	-0.84	-1.9
41	4.5	6.8
42	0.08	-1.3
43	-0.41	-0.30
44	-1.7	-1.5
45	-2.0	-1.9
46	-0.84	-0.07
47	-1.1	0.04
48	-1.6	-1.2
49	1.9	-4.1
50	-1.6	-1.8
51	-3.2	-3.8
52	-1.5	-1.5
53	-0.57	-0.79
54	-2.3	-5.2
55	0.68	-0.86
56	-0.9	0.26
57	2.1	2.7
58	-0.07	0.17
59	2.4	1.1
60	-0.73	-0.81
61	2.3	-6.8
62	1.0	-0.04
63	0.57	-0.18
64	1.1	-0.17
65	0.41	0.52
66	-0.9	-1.1
67	1.2	2.3
68	-1.7	-1.5

z-Scores ≥ 3 or ≤ -3 are bolded



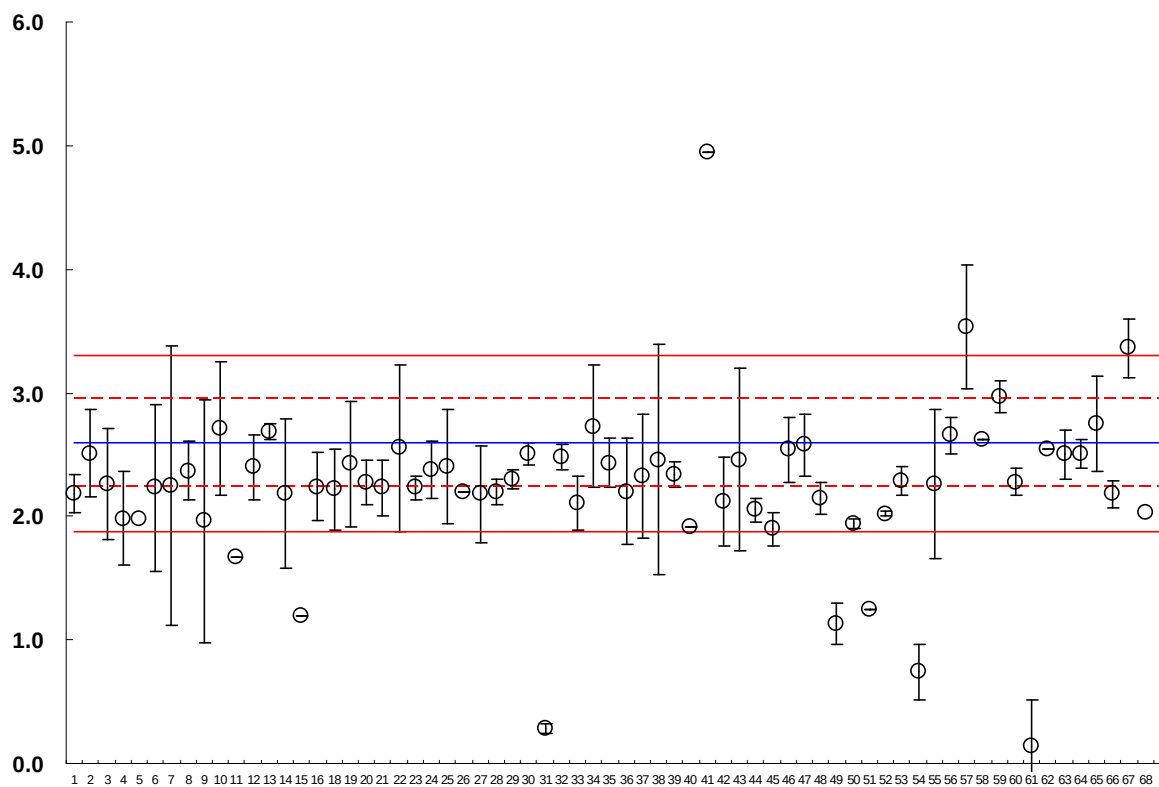
GRAPH I: Distribution of participants' reported concentration (mg kg⁻¹) for Sample I



Notes: Blue line represents the reference value (X_{ref}), red dotted lines represent $|z| = 1$, red solid lines represent $|z| = 2$ and error bars represent reported expanded uncertainty



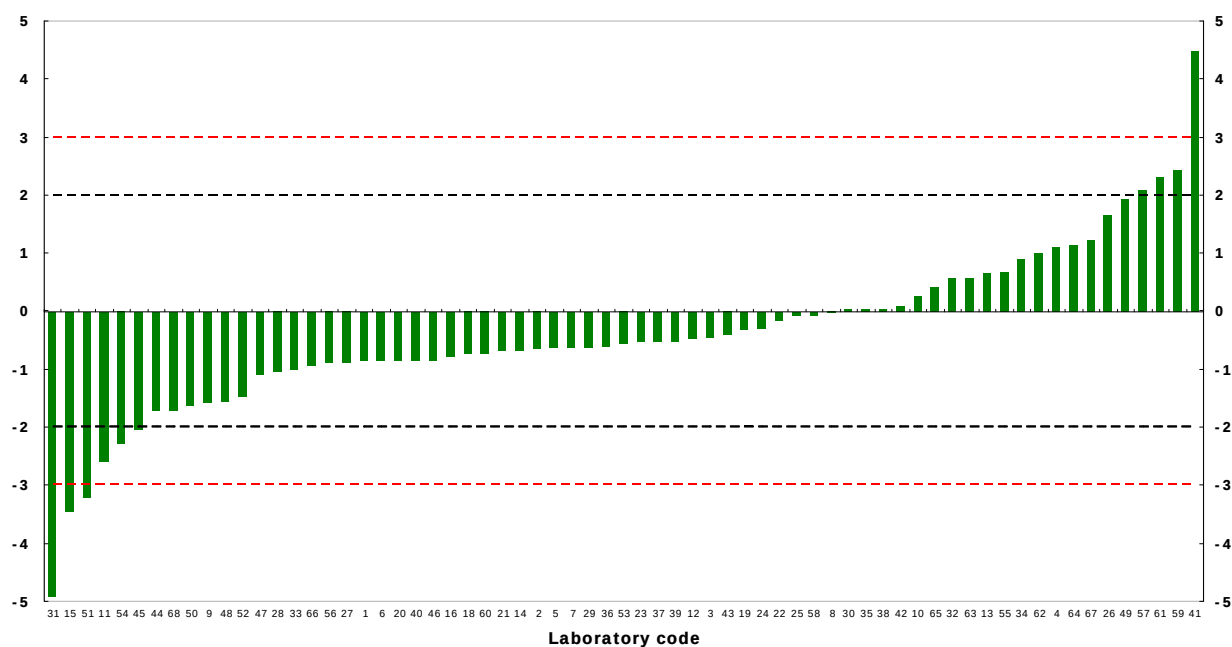
GRAPH II: Distribution of participants' reported concentration (mg kg⁻¹) for Sample II



Notes: Blue line represents the reference value (X_{ref}), red dotted lines represent $|z| = 1$, red solid lines represent $|z| = 2$ and error bars represent reported expanded uncertainty

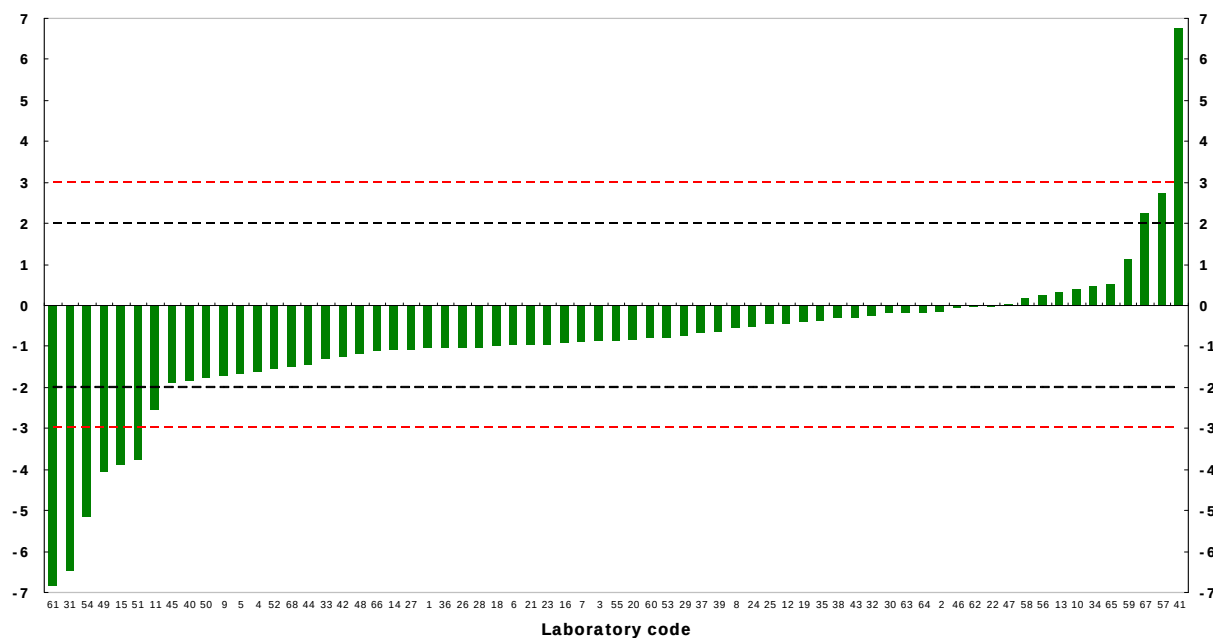


GRAPH III: Participants' z-scores for Sample I



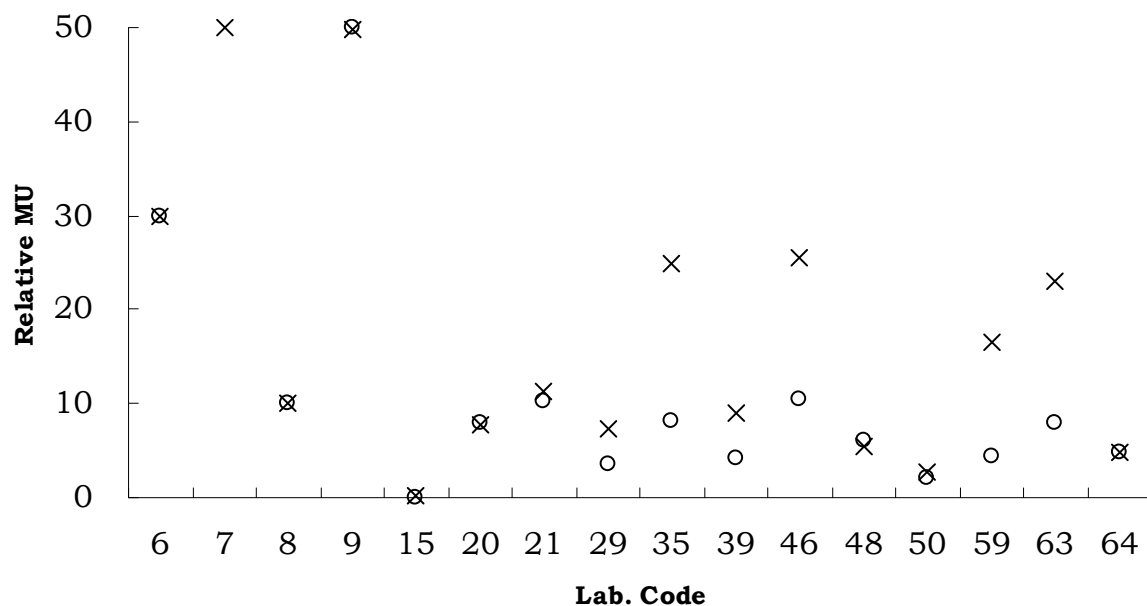


GRAPH IV: Participants' z-scores for Sample II





GRAPH V: Reported Relative Expanded Uncertainty by Accredited Laboratories (X for Sample I; O for Sample II)



**APPENDIX I: Homogeneity Test**

- a. A total of 11 bottles for Sample I and II were randomly selected for homogeneity test in April 2009. Two sub-samples (0.5 g) were taken from each bottle after reconstitution with distilled water. Determination of melamine was conducted using a validated LC-MS/MS method^{9.3}.
- b. Results of melamine concentration (mg kg⁻¹) in the sub-samples in the homogeneity tests were as follows:

Sample Code	Sample I		Sample Code	Sample II	
	Data 1	Data 2		Data 1	Data 2
1	1.13	1.14	3	2.29	2.29
7	1.20	1.17	17	2.50	2.25
13	1.14	1.14	21	2.42	2.45
26	1.16	1.16	38	2.45	2.45
37	1.15	1.12	43	2.39	2.46
45	1.15	1.15	52	2.44	2.43
59	1.16	1.15	66	2.39	2.46
63	1.21	1.14	72	2.45	2.40
76	1.18	1.15	85	2.44	2.42
81	1.15	1.16	89	2.48	2.39
99	1.19	1.18	97	2.46	2.37
Mean ($\bar{x}$)	1.16		Mean ($\bar{x}$)	2.41	
SD (S_x)	0.022		SD (S_x)	0.0645	
RSD (%)	1.9		RSD (%)	2.7	

- c. According to ISO13528:2005, the samples are considered to be homogeneous if:

$$S_s \leq 0.3\sigma_R \quad \text{where} \quad \begin{array}{ll} S_s & = \text{Between-sample standard deviation} \\ \sigma_R & = \text{Performance standard deviation} \end{array}$$

S_s is calculated as:

$$\sqrt{S_x^2 - (S_w^2 / 2)} \quad \text{where} \quad \begin{array}{ll} S_x & = \text{Standard deviation of sample averages} \\ S_w & = \text{Within-samples standard deviation} \end{array}$$

S_w is calculated as:

$$\sqrt{\sum w_t^2 / (2n)} \quad \text{where} \quad \begin{array}{ll} w_t & = \text{Absolute difference of the duplicate sample} \\ n & = \text{Number tested samples} \end{array}$$

**APPENDIX I (Cont'd): Homogeneity Test**

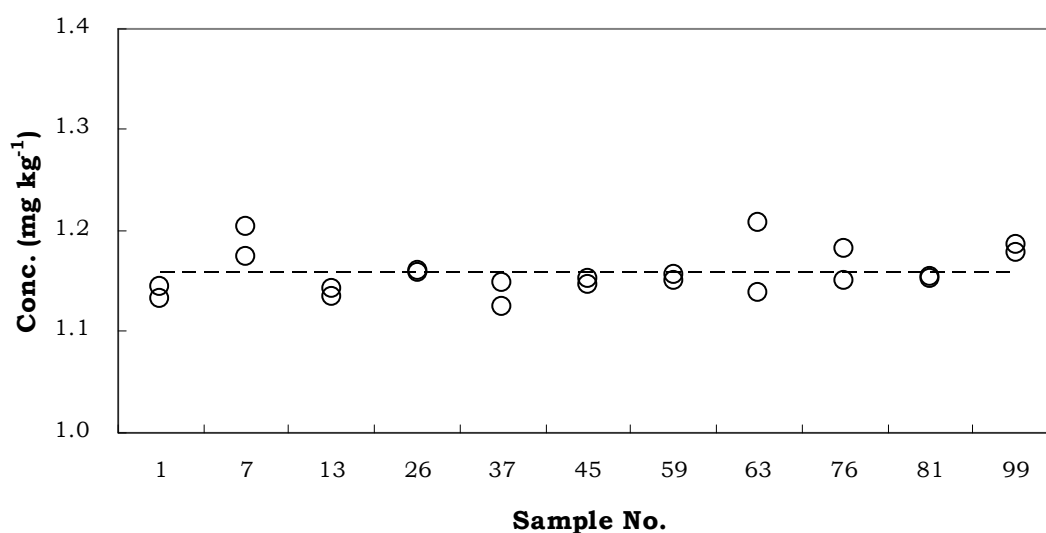
σ_R (in percent) is estimated using Horwitz equation of $2 C^{-0.15}$ where C was the mean concentration in mass fraction of melamine from homogeneity test:

		<u>Sample I</u>	<u>Sample II</u>
$\bar{x}$ (mg kg ⁻¹)	:	1.16	2.41
σ_R (%)	:	15.5	13.9
$0.3\sigma_R$ (%)	:	4.7	4.2
$0.3\sigma_R$ (mg kg ⁻¹)	:	0.054	0.10
Σw_t^2 (mg ² kg ⁻²)	:	0.0761	0.0928
S_w (mg kg ⁻¹)	:	0.0186	0.0649
S_x (mg kg ⁻¹)	:	0.0221	0.0648
S_s (mg kg ⁻¹)	:	0.0177	0.0457

Since the between-sample standard deviations (S_s) for Sample I and II in the homogeneity test for melamine were less than those of the corresponding value of $0.3\sigma_R$, the materials were considered homogeneous and should be adequate to be used in the PT program.

d. Distribution plot of duplicate analysis of the random samples:

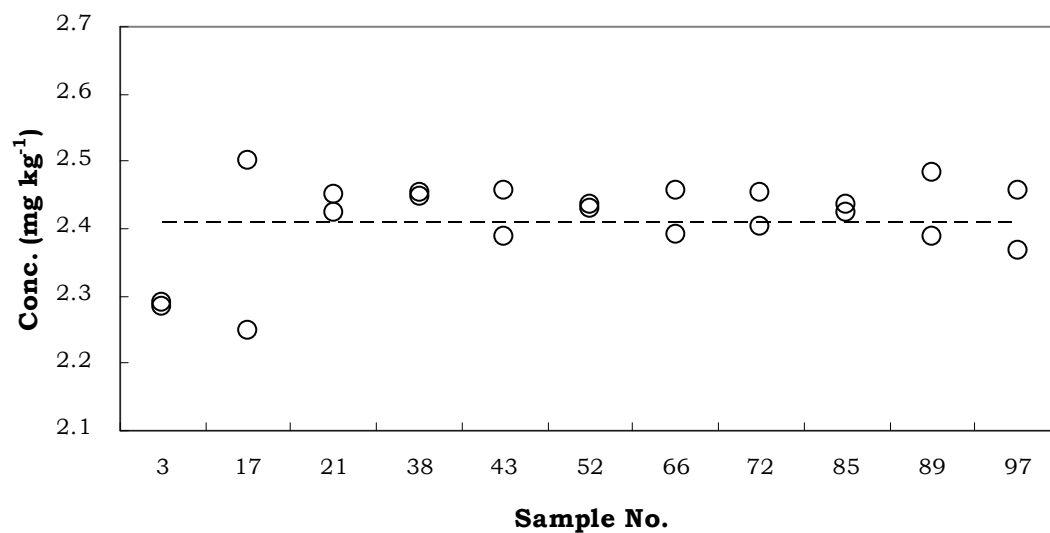
(i) Sample I





APPENDIX I (Cont'd): Homogeneity Test

(ii) Sample II



**APPENDIX II: Stability Test**

- a. Stability of melamine at room temperature of about 25 °C was monitored in July and October 2009.
- b. One sample was randomly taken and analyzed in triplicate under the same operational conditions as those in homogeneity test.
- c. Stability of samples was evaluated from the absolute difference between the mean value obtained in homogeneity test ($\bar{x}$) and the mean value ($\bar{y}_i$) of each stability test. According to ISO13528:2005, the absolute difference should not be more than $0.3\sigma_R$.

$$\text{ie. } |\bar{x} - \bar{y}_i| \leq 0.3\sigma_R$$

- d. Results of stability test for (i) Sample I and (ii) Sample II were summarized as follows:

(i)

Period	Melamine concentration (mg/kg)					$ \bar{x} - \bar{y}_i $	$0.3\sigma_R$	Status
	#1	#2	#3	$\bar{y}_i$	$\bar{x}$			
July 2009	1.15	1.12	1.18	1.15	1.16	0.01	0.05	Pass
October 2009	1.13	1.11	1.14	1.13	1.16	0.03	0.05	Pass

(ii)

Period	Melamine concentration (mg/kg)					$ \bar{x} - \bar{y}_i $	$0.3\sigma_R$	Status
	#1	#2	#3	$\bar{y}_i$	$\bar{x}$			
July 2009	2.46	2.38	2.43	2.42	2.41	0.01	0.10	Pass
October 2009	2.47	2.46	2.46	2.46	2.41	0.05	0.10	Pass

- e. Stability of melamine in the lyophilised samples was found to be satisfactory over the entire period of the PT program.



APPENDIX III: Instructions to Accreditation Bodies

INSTRUCTIONS (FOR ACCREDITATION BODIES)

1. ORGANIZERS

The PT program on “Melamine in Milk (APLAC T071)” is organized by the Hong Kong Government Laboratory (HKGL) in collaboration with Hong Kong Accreditation Service (HKAS) under the auspices of the Asia-Pacific Laboratory Accreditation Cooperation (APLAC). The main objectives of this proposal are to provide an analytical forum for participants in the quantitative analysis of melamine in fish feed sample and to evaluate the measurement capability of participating laboratories that providing such testing services.

2. SAMPLE

Accreditation bodies (AB) should receive the PT material(s) and instruction information (soft copy) from the organizers. Each PT material consists of **TWO** amber sample bottles, the blue labelled Test Sample I and the orange labelled Test Sample II, and each containing about 5 g of dried milk powder (size $\leq 200\ \mu\text{m}$). Upon receipt, AB should carefully check the samples (number of bottles, physical conditions, etc.) and shall promptly acknowledge the organizers by returning the “Receipt Form (for Accreditation Bodies)” electronically to T71@govtlab.gov.hk. New sample(s) will be replaced for any missing and damaged bottles.

AB shall distribute the **TWO** bottles (*please ensure it contains one Samples I bottle and one Sample II bottle*) to each of their nominated laboratories as soon as possible. The samples are recommended to be stored in a secure environment at room temperature (around 25°C) if immediate dispatch is not possible.

AB shall ask their nominated laboratories to check the samples carefully upon receipt and promptly acknowledge the organizers by returning the “Receipt Form (for Participating Laboratories)” electronically to T71@govtlab.gov.hk

3. RESULTS

AB shall remind their nominated laboratories to complete Part I and II of the “Result Proforma” and submit electronically to the organizers HKGL on or before 12 Sept 2009 at T71@govtlab.gov.hk. (Notes: 1. Under normal circumstances, results submitted after the deadline will not be accepted. 2. To avoid poor transmission quality, results by fax are generally not acceptable.)

4. CONTACT

Please contact the organizers for further enquiries at T71@govtlab.gov.hk

**APPENDIX IV: Instruction for Participating Laboratories****INSTRUCTIONS
TO PARTICIPATING LABORATORIES****1. ORGANIZERS**

The PT program on “Melamine in Milk (APLAC T071)” is organized by the Hong Kong Government Laboratory (HKGL) in collaboration with Hong Kong Accreditation Service (HKAS) under the auspices of the Asia-Pacific Laboratory Accreditation Cooperation (APLAC). The main objectives of this proposal are to provide an analytical forum for participants in the quantitative analysis of melamine in milk sample and to evaluate the measurement capability of participating laboratories that providing such testing services.

2. SAMPLE

- (a) Participating laboratory will be given **TWO** amber sample bottles, Test Sample I (blue label) and Test Sample II (orange label), and each containing about 5 g of dried milk powder (≤ 200 m) from his accreditation body (AB) or directly from the organizers.
- (b) Upon receipt of the samples, participating laboratory should carefully inspect the samples for any physical damages and defects. If presence of abnormality is detected, please contact the organizers at “T71@govtlab.gov.hk”. New sample(s) will be replaced if necessary.
- (c) If conditions of the samples are satisfactory, participating laboratory shall promptly acknowledge the receipt of the samples by returning the “Receipt Form (for Participating Laboratories)” electronically to the organizers at “T71@govtlab.gov.hk”.
- (d) Intact samples are recommended to be stored in a secure environment at room temperature (around 25°C) before use. Samples should be immediately capped after use and be kept in a dry box when not in use.

3. ANALYSIS

- (a) Participating laboratory is required to determine the concentration (in **mg kg⁻¹**) of melamine in the **TWO** sample bottles after reconstitution (follow the attached **Reconstitution Procedure**). The approximate concentration is in the range of 0.1 to 5 mg kg⁻¹.
- (b) Participating laboratory should use their test method (accredited, in-house, modified, ad hoc method, etc.) which is normally used for quantifying melamine for its customer. Details of the analytical method used should be given on the result sheet provided.



4. RESULTS

- (a) Report the tests results in Part I of the “Results Proforma”. If more than one determination has been performed, laboratories may also report all the test results together with the arithmetic mean in the form provided.
- (b) Estimate and report the expanded measurement uncertainty ($\pm$ mg kg⁻¹).
- (c) Fill in the information on the analytical method in Part II of the “Results Proforma”.
- (d) Submit the results electronically to the organizers on or before 12 Sept 2009 at T71@govtlab.gov.hk using the electronic copy of the “Result Proforma” provided.
(Notes: 1. Under normal circumstances, results submitted after the deadline will not be accepted. 2. To avoid poor transmission quality, results submitted by fax are generally not acceptable.)

5. CONTACT

Please contact the organizers at T71@govtlab.gov.hk for further enquiries.



APPENDIX V: Receipt Form for Accreditation Bodies

**RECEIPT FORM
(FOR ACCREDITATION BODIES)**

From: _____
(Name of Accreditation Body)

(Contact Person)

Email : _____

Date: _____

To: Dr. Yiu-chung WONG
Hong Kong Government
Laboratory, Hong Kong.

Email: T71@govtlab.gov.hk

I hereby acknowledge the receipt of _____ sample pairs (labelled as Sample I and Sample II) for the APLAC T071 program.

The sample(s) is/are found (damaged / satisfactory)* and should be (suitable / not suitable)* for laboratory testing.

Remark(s) : _____

Number of samples

* Please circle as appropriate



APPENDIX VI: Receipt Form for Participating Laboratories

RECEIPT FORM (FOR PARTICIPATING LABORATORY)
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From: _____
(Name of Participating Laboratory)

To: Dr. Yiu-chung WONG
The PT Advisory Board
Hong Kong Government
Laboratory, Hong Kong.

(Contact Person)

Email
: _____

Email: T71@govtlab.gov.hk

Date:

I hereby acknowledge the receipt of **TWO** samples (labeled as Sample I and Sample II) for the APLAC T071 program.

The sample(s) is/are found (damaged / satisfactory)* and should be (suitable / not suitable)* for laboratory testing.

Remark(s) : _____

* Please circle as appropriate



APPENDIX VII: Reconstitution Procedure

RECONSTITUTION PROCEDURE

1. Each bottle contains about 5 g of lyophilized milk powder, which is equivalent to at least 60 g of liquid milk.
2. Analysis of melamine should be performed using liquid milk, **NOT lyophilized powder**.
3. For the preparation of a 10 g test portion of liquid milk, 9.20 g of distilled water are added to 0.80 g of the lyophilized milk powder. The weighing should be accurate.
4. Well mix the sample with shaking.
5. Analysis should be conducted immediately after reconstitution and it is analogous to the analysis of liquid milk. Concentration determined in mg kg⁻¹ should be reported.

**APPENDIX VIII: Result Proforma****RESULTS PROFORMA**<Both Part I & II **MUST** be completed by Participating Laboratory>

Laboratory Code: _____ (official use)

Name of Participating Laboratory: _____

Contact Person / Email: _____

Signatory: _____

Part I: Analytical Results

Test Sample	Concentration of Melamine (in mg kg^{-1})				Measurement Uncertainty	
	#1	#2	#3	Mean	Expanded Uncertainty (mg kg^{-1})	Coverage Factor (k)
I						
II						

Note: If value determined is less than the limit of quantification (LOQ), please specify, eg. < 0.1 mg kg^{-1}

§Report values to a maximum of 3 significant figures or 3 decimal places, whichever is appropriate

¶It is the responsibility of the participants to avoid collusion and falsification of result so as to ensure a reliable assessment to be made in this proficiency testing program. Information on the identities and results of the participating laboratories will be kept confidential to the respective nominating accreditation bodies and the organizers (APLAC, HKAS and HKGL). If laboratories submit their results through their accreditation bodies, their results may be disclosed to and released through their accreditation bodies.

♦Please submit this “Result Proforma” electronically to the organizers at T71@govtlab.gov.hk

To be continued ...

**Part II: Method Information**

1. *Analytical Instrument: LC-UV / LC-MS / LC-MS/MS / GC-MS / ELISA / CE
Others (pls. specify):
- *Derivatization: YES _____ ; NO _____
 (pls. specify)
2. Chromatographic col.: eg. C₁₈ (150mm x 2.1mm, 5μm) or DB 5 (30m x 0.25mm x 25μm)

3. Mobile phase(s):
 or Carrier gas

4. *Calibration: External cal. curve; One point cal.; Standard addition;
Others (pls. specify):
5. *Internal standard YES (pls. specify) _____ ; NO _____

6. Extraction solvent(s):

7. *Extraction technique: Liq-liq. Extraction *(Agitation; Shaking; Ultrasonic)
SPE *(MCX, SCX, C₁₈, Florisil, Alumina, Others)
Others:

 (please specify)
8. Extraction duration: _____ hrs. _____ min.
9. Clean-up procedure

10. Recovery (%)

11. *Correction for recovery YES; NO
12. *Method accredited: YES; NO
13. Other additional information:

* Please circle as appropriate